Supplementary Material

Supplementary Methods

Demographic assessment

General demographic details were collected, along with complete disease-specific evaluation in medication "on" condition. Assessment of MSA patients was performed using the Multiple System Atrophy Rating Scale (UMSARS) parts I-II-IV[1], whereas PD patients were evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS) parts I-III. The Hoehn & Yahr (HY) scale[2,3] was employed to assess disease stage. Furthermore, patients underwent an abbreviated assessment (level I) of cognitive domains according to the Movement Disorder Society (MDS) Task Force[4], including the Mini-Mental State Examination (MMSE[5]), the Montreal Cognitive Assessment (MoCA[6]), the Clock Drawing Test (CDT[7]), and the Frontal Assessment Battery (FAB[8]). We used Italian-validated versions, and raw scores were adjusted for age, sex, and educational level according to established correction grids. The severity of depressive symptoms was evaluated through the Beck Depression Inventory (BDI)-II[9]. Other relevant data were collected: the levodopa equivalent daily dose (LEDD, according to[10]), the presence of hyposmia according to medical reports; probable REM sleep behavior disorder (pRBD) according to a cut-off score ≥ 6 in the RBD Screening Questionnaire (RBDSQ)[11], constipation (following Rome III criteria[12]) and urinary dysfunction, neurogenic orthostatic hypotension (nOH; based on specific prescription patterns and/or the detection during clinical examination of a fall of at least 20 mmHg in systolic blood pressure (30 mmHg in patients with supine hypertension) and/or 10 mmHg in diastolic blood pressure within 3 min of standing[13]).

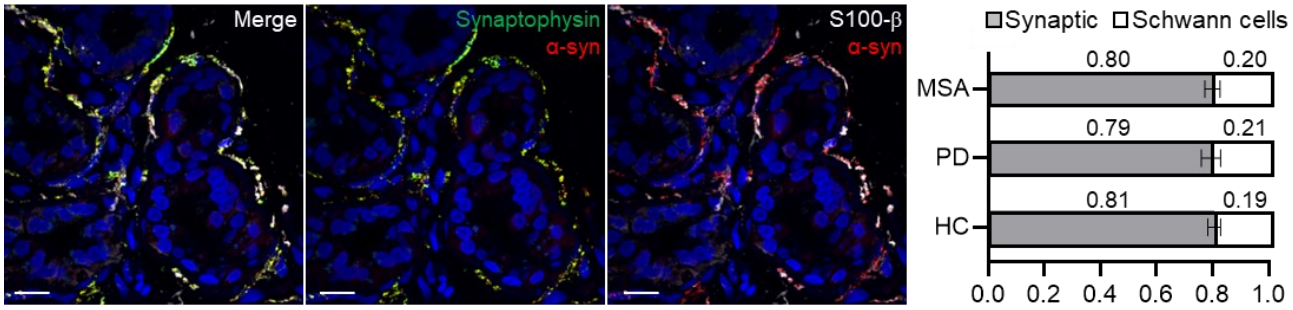
Immunofluorescence

To verify total α-synuclein distribution, immunofluorescences were performed by ON incubation at RT with the following primary antibodies diluted in PBS plus 0.1% Triton™ X-100: rabbit anti-α-synuclein (SynS3062, 1:1000); mouse anti-synaptophysin (Dako, clone DakSynap, 1:200) as synapse marker, and chicken anti-S100β (Synaptic System, 1:700). Mix of secondary antibodies containing donkey anti-mouse Alexa 488, donkey anti-rabbit Alexa 568 and donkey anti-chicken 647 (Jackson Immunoresearch, 1:300) for 2 hours at 37°C in the dark. Nuclei were counterstained with Hoechst 33342 (ThermoFisher; 1:5000 in PBS, 10 min) and sections mounted using Mowiol®+DABCO®.

Supplementary Table 1: Skin biopsy measures in MSA-P *vs* MSA-C patients. Comparisons were performed using the t-test for independent samples or the Mann-Whitney test as appropriate.

Variable	MSA-P (N = 16)	MSA-C (N = 15)	*p*-value
Sudomotor autonomic compartment			
Synaptic density	18.81 ± 10.25	19.91 ± 9.58	0.760
Synaptic AS-PLA	7.63 ± 6.90	5.38 ± 4.30	0.553
PLA score	157.59 ± 175.45	114.46 ± 100.56	0.453
Schwann cell density	15.16 ± 8.77	15.07 ± 10.48	0.906
Glial AS-PLA	2.89 ± 2.77	2.84 ± 3.42	0.706
Synaptic AS-PLA – Glial AS-PLA	4.74 ± 6.70	2.53 ± 3.47	0.252
Somatic cutaneous nerve compartment			
Schwann cell density	0.25 ± 0.07	0.26 ± 0.10	0.776
Glial AS-PLA	3.07 ± 3.04	2.58 ± 1.77	0.965

Supplementary Figure 1. Total α-synuclein (a-syn, in red) distribution between synaptic (Synaptophysin, green) and Schwann cell (S100-β, in white) compartments, in the autonomic structures. Nuclei are counterstained with Hoechst (blue). Scale bar = 10 μm. The graph indicates the fraction of α-synuclein within synaptic terminals (grey) and Schwann cells (white) relative to total α-synuclein of MSA, PD and HC. Values are expressed as mean ± SD. No difference is observed among the three cohorts.



Supplementary Table 2: Multivariable regression analyses of skin biopsy measures in the sudomotor autonomic and somatic cutaneous nerve compartments.

Outcome variable sudomotor autonomic compartment	Model	Predictor	B (95% CI)	β	*p*-value
Synaptic AS-PLA	Model 1	MSA	0.508 (0.219-0.797)	0.549	**0.001**
		PD	0.438 (0.137-0.739)	0.452	**0.005**
	Model 2	MSA	0.108 (-0.104-0.319)	0.150	0.311
PLA score	Model 1	MSA	0.494 (0.169-0.819)	0.489	**0.003**
		PD	0.437 (0.099-0.776)	0.414	**0.012**
	Model 2	MSA	0.120 (-0.120-0.361)	0.149	0.320
Glial AS-PLA	Model 1	MSA	0.243 (-0.172-0.659)	0.258	0.245
		PD	0.158 (-0.272-0.589)	0.158	0.462
	Model 2	MSA	0.086 (-0.276-0.449)	0.089	0.632
Synaptic AS-PLA – Glial AS-PLA	Model 1	MSA	0.198 (-0.120-0.516)	0.230	0.218
		PD	0.264 (-0.077-0.604)	0.290	0.126
	Model 2	MSA	0.008 (-0.243-0.260)	0.010	0.947
Outcome variable **cutaneous nerve compartment**	**Model**	**Predictor**	**B (95% CI)**	**β**	***p*-value**
Glial AS-PLA	Model 1	MSA	0.049 (0.082-0.816)	0.473	**0.017**
		PD	0.383 (-0.022-0.788)	0.363	0.063
	Model 2	MSA	0.233 (-0.085-0.551)	0.246	0.146

Only measures with statistically significant differences in overall between-group comparisons are reported. Model 1 included the whole cohort and was adjusted for age and sex; HC group was used as the reference category. Model 2 included only patients with MSA and PD and was adjusted for age, sex, and disease duration; PD was used as the reference category. Outcome variables were log-transformed before analysis. Unstandardized B coefficients with 95% confidence intervals and standardized β coefficients are reported; significant *p*-values are highlighted in bold.

Supplementary Bibliography

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